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***Interpretive Summary***

Environmental and economic sustainability of milk protein production in dairy cattle requires reducing nitrogen intake while maximizing post rumen flow of amino acids. Byproducts from human food production can provide key nutrients for rumen microbial populations. The objective of this study was to evaluate the effects of a commercial fermentation byproduct on omasal flow of amino acids from non-microbial, bacterial, and protozoal flows. Observed digestion parameters were compared against predictions from a mathematical model. Results indicate that fermentation byproduct can be successfully used to increase post rumen flow of amino acids while maintaining high levels of rumen microbial activity.

**Rumen digestion kinetics, microbial yield, and omasal flows of non-microbial, bacterial and protozoal amino acids in lactating dairy cattle fed fermentation byproduct or urea as a soluble nitrogen source.**

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***Abstract***

The objective of this study was to evaluate the effect of a fermentation byproduct on rumen function, microbial yield and composition and flows of nutrients from the rumen in high producing lactating dairy cattle. Eight ruminally cannulated multiparous Holstein cows averaging (mean ± SD) 60 ± 10 days in milk and 637 ± 38 kg of body weight were randomly assigned to one of two treatment sequences in a switchback design. Treatment diets contained (dry matter basis) 44% corn silage, 13% alfalfa silage, 12% ground corn, and 31% protein premix containing either a control mix of urea and wheat middlings (**CON**) or a commercial fermentation byproduct meal (Fermenten™, Arm and Hammer Animal Nutrition, Princeton, NJ) at 3% diet inclusion rate (**EXP**). The trial consisted of three 28 d experimental periods, where each period consisted of 21 d of diet adaptation and 7 d of data and sample collection. A triple-marker technique and doubly-labeled $^{15}N^{15}N$-urea were used to were used to measure protozoal, bacterial, and non-microbial omasal flow of AA. Rumen pool sizes and omasal flows were used to determine digestion parameters, including fractional rates of carbohydrate digestion, microbial

growth, and yield of microbial biomass per gram of degraded substrate. Fermentation byproduct inclusion in EXP diets increased microbial N and amino acid N content in microbes relative to microbes from CON cows fed the urea control. Microbial amino acid profile did not differ between diets. Daily omasal flows of AA were increased in EXP cows as a result of decreased degradation of feed protein. The inclusion of the fermentation byproduct increased non-microbial AA flow in cows fed EXP vs. CON. Average protozoa contribution to microbial N flow was 16.8%, yet protozoa accounted for 21% of the microbial AA flow, with a range of 8 to 46% for individual AA. Cows in this study maintained an average rumen pool size of 320 g of microbial N and bacterial and protozoal pools were estimated at 4 different theoretical levels of selective protozoa retention. Fractional growth rate of all microbes was estimated to be 0.069 $h^{-1}$, with a yield of 0.44 g microbial biomass per g of carbohydrate degraded. Results indicated that fermentation byproduct can increase omasal flow of AA while maintaining adequate rumen N available for microbial growth and protein synthesis. Simulations from a developmental version of the Cornell Net Carbohydrate and Protein System indicated strong agreement between predicted and observed values, with some areas key for improvement in AA flow and bacterial vs. protozoal N partitioning.

**Keywords**: rumen protozoa, amino acids, microbial growth, CNCPS, Fermenten

## INTRODUCTION

Byproducts of human food production have successfully been used to improve the sustainability of the dairy industry (VandeHaar and St-Pierre, 2006). Efficient and effective use of byproduct feeds requires adequate knowledge of the fermentation characteristics of the feed (Fessenden and Van Amburgh, 2016). Fermenten™ (Church & Dwight, Inc., Princeton, NJ) is a commercially available fermentation byproduct feed derived from glutamic acid production and

contains high amounts of rumen available nitrogen compounds in the form of soluble AA and small peptides (Fessenden, 2016). Lean et al. (2005) reported an increase in microbial protein flow from continuous fermenters fed fermentation byproduct. The authors attributed this increase in microbial biomass to stimulation of microbial protein synthesis by small peptides and AA (Cotta and Russell, 1982). However, experiments on the same byproducts in vivo have not demonstrated consistent production responses (Broderick et al., 2000, Penner et al., 2009). In a companion paper, Fessenden et al. (20XXa) demonstrated that fermentation byproduct decreased dietary protein degradation in the rumen by approximately 15%, indicating a possible sparing effect of degradable protein through and unknown mechanism. The results from the first portion of the study warranted further investigation into possible effects on omasal AA flow, the partition of N flows between microbial and non-microbial fractions, and the effects on microbial growth and digestion parameters.

Mathematical models such as the Cornell Net Carbohydrate and Protein system (**CNCPS**) (Higgs et al., 2015, Van Amburgh et al., 2015) have been successfully used to optimize rumen microbial output and meet animal nutrient requirements while reducing N losses to the environment (Tylutki et al., 2008). A new, dynamic and more mechanistic version of the CNCPS was developed (Higgs, 2014) that describes rumen degradation of substrates with mechanistic representations of growth of bacteria and protozoa and includes interactions among protozoa and bacteria such as predation and intra-ruminal microbial N turnover. Evaluations of this model indicated a strong ability to predict the partitioning between microbial and non-microbial nitrogen flows; however the partitioning between protozoa and bacteria along with individual AA predictions might require further refinement.  As with most model development, evaluations

of the rumen sub-model with independent data can be helpful for determining areas for improvement.

The hypothesis of this study was that the decreased ruminal protein degradation associated with fermentation byproduct inclusion would increase total AA flow at the omasal canal, with limited effects on bacteria and protozoa growth and turnover. This hypothesis was based off the previous findings regarding microbial and non-microbial N flows reported in the companion paper (Fessenden et al., 20XXa). The objectives of this study were to 1) evaluate the effect of urea with wheat midds or commercial fermentation byproduct on omasal flows of non-microbial, bacterial, and protozoal flows of AA, and 2) provide comparisons of model predicted vs. measured values for rumen microbial digestion and growth parameters.

## MATERIALS AND METHODS

The experiment was conducted from April to July 2014 at the Cornell University Ruminant Center in Harford, NY. All animals involved in this experiment were cared for according to the guidelines of the Cornell University Animal Care and Use committee. The committee reviewed and approved the experiment and all procedures carried out in the study.

### *Animals, Treatments and Experimental Design*

Eight ruminally cannulated multiparous Holstein cows averaging (mean ± SD) 60 ± 10 d in milk and 637 ± 38 kg of body weight were enrolled in the study. All cows were allowed a 3 wk pre-trial acclimation period where animals were managed and housed in a tie-stall and individually fed a common diet. Cattle were then stratified by pre-trial milk production and randomly assigned to one of two treatment sequences in a switchback design with three 28 d periods. Each period contained 21 d for diet adaptation and 7 d of data and sample collection.

Treatment diets contained (DM basis) 44% corn silage, 13% alfalfa silage, 12% ground corn, and 31% protein premix containing either a control mix of urea and wheat middlings (**CON**) or Fermenten (**EXP**) at 3% inclusion rate in the final diet (Table 1). All cattle received both diets throughout the three experimental periods according to their randomly assigned sequence of either EXP-CON-EXP or CON-EXP-CON (switchback design). Diets were formulated using CNCPS v. 6.5 (Van Amburgh et al., 2015). Full details of the cattle housing, milking, and feeding management are described in Fessenden et al. (20XXa).

***Sample Collection and Processing***

Digesta flow leaving the rumen was quantified using the omasal sampling technique developed by Huhtanen et al. (1997) and adapted by Reynal and Broderick (2005). A triple marker system using CoEDTA (Udén et al., 1980), $YbCl_3$ (modified from Siddons et al., 1985), and undegraded aNDFom (**uNDFom**) (Raffrenato et al., 2018) was used to quantify liquid, small particle, and large particle flow at the omasal canal, respectively. Double-labeled urea ($^{15}N^{15}N$-urea, 98% purity, Cambridge Isotope Laboratories Inc., Andover, MA) was infused into the jugular vein for use as a microbial marker following the method used for studies on urea recycling (Lobley et al., 2000). Details on marker preparation and infusion are reported in Fessenden et al. (20XXa).

Samples of whole omasal contents were collected from the omasal canal at 2 h intervals during three 8 h sessions: at 16:00, 18:00, 20:00, and 22:00 h on day 24; at 00:00, 02:00, 04:00, and 06:00 h on day 26; and at 08:00, 10:00, 12:00, and 14:00 on day 27. Sampling times were chosen to encompass every 2 h of the average 24 h feeding cycle. During each 8 h session, a 425 mL spot sample was obtained at the first 3 time points, while 675 mL were taken at the last time point. Spot samples were split into subsamples of 50 mL (x2), 125 mL, and 200 mL; with an

additional 250 mL subsample at the last time point. The 50 mL subsamples were used for a separate study of nutrient flows (Fessenden et al., 20XXa). The 125 mL subsamples were held on ice and combined within session, yielding a 500 mL sample for bacterial isolation. The 200 mL samples were combined within period and stored at −20 °C, yielding a 2.4 L composite for digestion phase separation. The additional 250 mL sample obtained at the end of each session was strained through 2 layers of cheesecloth and immediately processed to isolate protozoa (described later).

The 2.4 L pooled omasal composites were thawed and separated into omasal large particle (**LP**), small particle (**SP**) and liquid phase (**LQ**) as described in the companion paper (Fessenden et al., 20XXa). All phase samples were freeze dried and either ground through a 1 mm screen on a Wiley mill (LP) or homogenized with a mortar and pestle (SP and LQ) before analysis. Concentrations of Yb, Co, and uNDFom in each phase were used to calculate the concentration of each nutrient in a theoretical entity representing omasal true digesta (**OTD**) (France and Siddons, 1986). As such, the reported flows and concentrations of any given nutrient in OTD is a mathematical calculation based on re-constitution factors determined using the triple marker technique and measured values of the nutrient in LQ, SP, and LP. This mathematical construct is referred to in this paper as OTD. On the last day of each period, rumen contents were evacuated, weighed, mixed, and a representative sample was obtained for pool size determinations and stored at −20 °C prior to lyophilization and determination of rumen nutrient pool sizes.

The bacterial isolations from each 8 h sampling period were combined within period to yield an omasal bacteria sample for each cow within period. Microbial isolation was performed according to Whitehouse et al. (1994) with modifications. Briefly; whole omasal contents were filtered through 4 layers of cheesecloth and solids were rinsed once with saline, and the filtrate

(I) was treated with formalin (0.1% v/v in final solution) and stored at 4 °C. The solids retained on the cheesecloth were incubated for 1 h at 39 °C in a 0.1% methylcellulose solution, mixed for 1 min at low speed (Omni Mixer, Omni International, Kennesaw, GA) to detach solids associated bacteria, and held at 4 °C for 24 h. The contents were then squeezed through 4 layers of cheesecloth and the filtrate (II) was treated with formalin (0.1% v/v in final solution). Filtrates I and II were held at 4 °C until the end of the sampling period, then combined and centrifuged at 1000 x *g* for 5 min at 4 °C to remove small feed particles. The supernatant was centrifuged at 15,000 x *g* for 20 min at 4 °C and the bacterial pellet, representing both solid and liquid associated bacteria, was collected and stored at −20 °C until lyophilization and later analysis.

Protozoa were isolated from whole omasal contents using the procedure described by Denton et al. (2015) with modifications (Figure 1). Strained omasal fluid (250 mL) was combined 1:1 with pre-warmed, anaerobically prepared Simplex type buffer and added to a pre-warmed separatory funnel. Plant particles were removed by aspiration after 1 h of incubation at 39 °C, allowing for removal of 50 mL of fluid to a pre-calibrated 450 mL line on the funnel. Funnel contents were then preserved with formalin (0.1% v/v in final solution) and stored for < 4 d at 4 °C. At the end of each sampling period, preserved contents were centrifuged at 1000 x *g* for 5 min at 4 °C, the pellet was re-suspended in saline, and protozoa were isolated on a nylon cloth with a 20 μm pore size (14% open area, Sefar, Buffalo, NY). The protozoa isolate was washed with saline (500 mL) to reduce bacterial contamination. Microscopic inspection of the retained protozoa and filtrate indicated low feed contamination and good recovery of small protozoa. After isolation, protozoa were stored at −20 °C, followed by lyophilization and measurement of DM amount to calculate yield of protozoal DM per L of omasal fluid (Ahvenjärvi et al., 2002).

***Sample Analysis***

177 Samples of freeze-dried bacteria, protozoa, omasal fractions and rumen contents were
178 analyzed for residual DM after 6 h at 105 °C and ash according to AOAC (2005). Total N was
179 determined using a combustion assay (Leco FP-528 N Analyzer, Leco Corp., St. Joseph, MI).
180 Samples were analyzed for non-ammonia nitrogen (**NAN**) and $^{15}N$ as follows: 20 μg of N from
181 each sample was weighed into tin capsules and 10 μL of 72 m*M* $K_2CO_3$ were added and
182 incubated at 60°C overnight to volatilize ammonia. Samples were then analyzed for NAN and
183 $^{15}N$ using a Carlo Erba NC2500 elemental analyzer interfaced with an isotope ratio mass
184 spectrometer (Cornell University Stable Isotope Laboratory, Ithaca, NY).

185 Amino acid content of bacteria, protozoa, and omasal fractions was determined by HPLC.
186 For all AA excluding Met, Cys, and Trp, sample containing 2 mg N was weighed into hydrolysis
187 tubes with 25 μL of 250 mM Norleucine as an internal standard. Samples were then hydrolyzed
188 at 110 ºC for 21 h in a block heater (Gehrke et al., 1985) with high-purity 6 M HCl (5 mL) after
189 flushing with $N_2$ gas. For Met and Cys, aliquots containing 2 mg N and the internal standard
190 were preoxidized with 1 mL performic acid (0.9 mL of 88% formic acid, 0.1 mL of 30% $H_2O_2$
191 and 5 mg phenol) for 16 h at 4 °C prior to acid hydrolysis as described above (Mason et al.,
192 1980, Elkin and Griffith, 1984). After hydrolysis, tube contents were filtered through Whatman
193 541 filter paper and filtrate was diluted to 50 mL in a volumetric flask with HPLC grade $H_2O$.
194 Aliquots (0.5 mL) were evaporated at 60 °C under constant $N_2$ flushing, with 3 rinses and re-
195 evaporations with HPLC grade $H_2O$ to remove acid residues. After final evaporation, hydrolysate
196 was dissolved in 1 mL of Na diluent (Na220, Pickering Laboratories, Mountain View, CA).

197 Individual AA hydrolysates were separated using an Agilent 1100 series HPLC (Agilent
198 Technologies, Santa Clara, CA) fitted with a sodium cation exchange column (Cat. no
199 1154110T, Pikering Laboratories, Mountain View, CA) using a 4 buffer step gradient and

column temperature gradient. Detection of separated AA was performed at 560 nm following post-column ninhydrin derivation. Standards (250 nM/mL) for the individual amino acids were prepared by diluting a pure standard in sample buffer. The volume of sample and standards loaded onto the column was 10 μL. For Trp determination, a separate aliquot of sample containing 2 mg N was hydrolyzed with 1.2 g of $Ba(OH)_2$ at 110 °C for 16 h on a block heater according to the method of Landry and Delhaye (1992). Included in the hydrolysis was 125 μL of 5-Methyl-Trp (5 m*M*) as an internal standard. After cooling to precipitate barium ions, an aliquot (3 μL) of the hydrolysate was added to 1 mL of acetate buffer (0.07 *M* sodium acetate) and analyzed using fluorescence detection (excitation = 285 nm, emission = 345 nm) after HPLC separation.

***Calculations***

Calculation of $^{15}N$ atom percent excess (**APE**) in rumen contents, omasal fractions and microbial samples; omasal nutrient flow, and partitioning of NAN are described in the companion paper (Fessenden et al., 20XXa). Briefly, total N entering the omasal canal was partitioned into three fractions: ammonia N, microbial N, and non-ammonia non-microbial N (**NANMN**). Total NAN flow was calculated as the difference between total N and ammonia N.

The N content of bacteria and protozoa was used to calculate flow of OM and DM in each microbial fraction. Partitioning of the microbial pool into bacterial and protozoal pools takes into account the differences in $^{15}N$ APE between bacteria and protozoa samples, therefore reducing the underestimation bias introduced by assuming bacterial $^{15}N$ enrichment as representative of all microbial biomass (Brito et al., 2007). Protozoal predation was estimated using the $^{15}N$ enrichment of the microbial fractions in the following manner

$$\text{Engulfed bacterial N} = \frac{\text{protozoa N flow } (g/d) \times \text{protozoa } ^{15}\text{N APE } (g/g) \times 0.9 \,/\, 0.5}{\text{bacteria } ^{15}\text{N APE } (g/g)}$$

In the preceding calculation, it is assumed that 90% of the enriched $^{15}$N in the protozoa is of bacterial origin; recognizing the capability of protozoa for de-novo synthesis of AA from ammonia (Williams and Harfoot, 1976; Williams and Coleman, 1997; Newbold et al., 2005). The calculation also assumes that 50% of the engulfed N is incorporated into cell N (Hristov and Jouany, 2005), an assumption also incorporated into a dynamic version of the CNCPS (Higgs, 2014). Protozoa consumption of bacterial DM and OM was determined using the N and OM content of the omasal bacteria.

Rumen OM, fermentable carbohydrate (**CHO**), NAN, and microbial NAN pool sizes were determined from nutrient analysis of the samples taken during the rumen evacuations. Measured $^{15}$N APE of the total rumen NAN pool was used to partition microbial and non-microbial N in the same manner as described for omasal NAN flows. Pool size calculations for digestible OM and CHO are as follows:

Digestible OM (kg) = Rumen OM (kg) – Microbial OM pool (kg) – rumen uNDFom pool (kg)

Digestible CHO (kg) = Rumen digestible OM pool (kg) - (Rumen CP pool – Microbial CP pool) – (rumen DM pool * diet fat content (g/g of DM))

To estimate the partition of the rumen microbial N pool into bacteria and protozoal pools, relative flows of bacteria and protozoa were multiplied by a factor representing selective retention of protozoa in the rumen. Reported rumen protozoa N retention in rumen vs. post-ruminal measurements vary widely, and range from < 5 % (Sylvester et al., 2005) to over 70% (Punia et al., 1992). Therefore, rumen protozoa $^{15}$N proportion of the total rumen $^{15}$N pool (**PP**)

was calculated at 4 different levels, from no selective retention to 75 % selective retention. To make this estimation, the ratio of protozoa 15N flow to total omasal 15N flow was divided by 1, 0.75, 0.5, and 0.25, representing selective retention of 0, 25, 50, and 75%, respectively:

Protozoa $^{15}$N proportion of the total rumen $^{15}$N, (PP; g/g) = [Protozoa $^{15}$N flow (g/d) / OTD $^{15}$N flow (g/d)] / (1,0.75,0.5,0.25)

The protozoa proportion of the rumen $^{15}$N at each of the 4 levels of selective retention, along with the APE of rumen contents and the microbial fractions were then used to calculate the rumen pool sizes for bacteria, protozoa, and total microbial NAN:

Protozoa NAN pool size (g) = [Rumen contents $^{15}$N APE (g/g) x Rumen total N (g) x PP, (g/g)] / protozoa $^{15}$N APE (g/g)

Bacteria NAN pool size (g) = [Rumen contents $^{15}$N APE (g/g) x Rumen total N (g) x (1-PP, g/g)] / bacteria $^{15}$N APE (g/g)

Microbial NAN pool size (g) = Protozoa NAN pool size (g) + Bacteria NAN pool size (g)

The value obtained when using a selective retention rate of 25% was used in calculations requiring a total rumen microbial pool size. Justification for this approach is discussed later in this paper. Rumen pool size and omasal flow was then used to calculate the fractional growth rate of total microbial, bacteria and protozoa fractions:

$$\text{Fractional growth rate } (h^{-1}) = \frac{\text{flow of microbial, bacterial, or protozoal N, g/h}}{\text{Rumen pool size of microbial, bacterial, or protozoal N g}}$$

Since flows and pool sizes were measured values, the fractional growth rate accounts for lyses and turnover in the rumen. The same pool size and flow approach was used to calculate the absolute and fractional degradation rates of OM and CHO in the rumen, where the numerator

was the hourly rate of disappearance of OM or CHO, and the denominator is the rumen pool size of digestible OM or CHO. Fractional rate of CHO degradation and fractional rate of microbial growth was then used to calculate yield of microbial cells per gram of CHO degraded ($\mathbf{Y_g}$)

$$Y_g \text{ (g cell DM / g CHO degraded)} = \frac{\text{fractional rate of microbial growth}}{\text{fractional rate of CHO degradation}}$$

Flows of individual AA in OTD was calculated using the concentration of AA in each omasal fraction and the triple marker system described in Fessenden et al (20XXa). Bacteria, protozoa, total microbial, and non-microbial AA flow were then calculated as follows:

Bacterial AA flow (g/d) = bacteria N flow (g/d) × bacteria AA (g/g N)

Protozoa AA flow (g/d) = protozoa N flow (g/d) × protozoa AA (g/g N)

Microbial AA flow (g/d) = protozoa AA flow (g/d) + bacteria AA flow (g/d)

Non-microbial AA flow (g/d) = OTD AA flow (g/d) – microbial AA flow (g/d)

At the end of the experiment, all relevant farm, cattle, and diet information were entered into an experimental version of the CNCPS v. 7 (Higgs, 2014) to provide comparisons with reported values. The model mechanistically describes substrate degradation using rates of passage and degradation (Waldo et al., 1972), and relates microbial growth to substrate availability (Russell et al., 1992) with modifications (Higgs, 2014). Protozoa, endogenous N transactions, N recycling, and large intestine degradation of substrate are all represented in a mechanistic manner. To provide the model with rates of CHO degradation, feedstuffs were analyzed using methods available from commercial laboratories. The fermentation time points used to calculate the rates of digestion for aNDFom were 30, 120 and 240 h (Raffrenato et al., 2018). Starch disappearance after 7 h of in vitro rumen incubation was used to calculate a fractional rate of

degradation (Fessenden 2011; Sniffen and Ward 2011). Comparisons were made of model predicted vs. measured values for substrate degradation, microbial growth, and post-ruminal AA flows.

### *Statistical Analyses*

All data were analyzed using SAS version 9.3 (SAS Institute Inc. Cary, NC). The same model as described in Fessenden et al. (20XXa) is reproduced here:

$$Y_{ijkl} = \mu + S_i + C_{j:i} + P_k + T_l + ST_{il} + \varepsilon_{ijkl}$$

where $Y_{ijkl}$ = dependent variable, $\mu$ = overall mean, $S_i$ = fixed effect of sequence i, $C_{j:i}$ = random effect of cow within sequence, $P_k$ = fixed effect of period k, $T_l$ = fixed effect of treatment l, $ST_{il}$ = fixed interaction effect of sequence i and treatment l, and $\varepsilon_{ijkl}$ = residual error. Degrees of freedom were calculated using the Kenward-Roger option. Means were determined using the least squares means statement, and treatment means were compared using the PDIFF option. Statistical significance was considered at $P \leq 0.05$ and trends were considered at $0.05 < P \leq 0.10$.

## RESULTS AND DISCUSSION

### *Microbial Nutrient Composition*

The OM content of omasal bacteria and protozoa did not differ between diets, and averaged 84.1 and 87.6%, respectively (Table 2). Organic matter content was similar to values obtained previously from rumen and omasal isolates (Brito et al., 2006; 2007), although OM content is strongly influenced by the isolation procedures used (Martin et al., 1994). Nitrogen content (% of DM) of bacteria and protozoa were both affected by diet, with increased N content in microbial isolates from cows fed EXP. When expressed on an OM basis, protozoa N did not differ between cows fed EXP or CON. This increased N content in bacteria could arise from several possible

mechanisms. With decreased protein degradation in cows fed EXP as reported in Fessenden et al. (20XXa), it is possible that less protein was degraded completely to ammonia, thus increasing AA and peptides available for microbial incorporation, leading to increased N content. Brito et al. (2007) reported a similar 5% increase in NAN content of fluid associated bacteria when urea vs. true protein supplements were fed to lactating dairy cows. An alternative possibility is a change in microbial reserve carbohydrate synthesis, resulting in more glycogen to dilute the measured NAN value. In microbial competition studies for substrate, Denton et al. (2015) demonstrated that protozoa sequestered up to 60% of available glucose and stored it as glycogen, while less than 2% was recovered in bacteria. In this study, cows fed CON demonstrated lower microbial N content, which could indicate more reserve CHO synthesis. This could also provide a possible explanation for the relative change in bacteria vs. protozoa NAN content when expressed on an OM basis. Bacteria N (% OM) increased by 3.5% (9.45 vs. 9.79% of OM for cows fed CON vs. EXP, respectively), while protozoa NAN content did not differ between diets. Differential amounts of glycogen synthesis by bacteria vs. protozoa could lead to the observed result. Omasal protozoa NAN content has been reported as low as 2.3% of DM (Brito et al., 2006), although sucrose was used in the isolation procedure, likely influencing N content. The protozoa isolation method employed in the current study deliberately omitted any addition of glycogenic compounds to avoid biasing the N measurement. Further examination into the rate and extent of uptake of AA and glucogenic precursors by microbial communities might be warranted. Enrichment of $^{15}N$ in bacteria and protozoa was similar for cows fed CON vs. EXP. The mean protozoal:bacterial $^{15}N$ enrichment ratio of 0.62 was within the range of values reported in the literature: 0.40 (Ahvenjärvi et al., 2002), 0.63 (Hristov and Broderick, 1996), 0.75 (Cecava et al., 1991). Some authors attributed low enrichment to feed contamination in the

331 isolation method, although $^{15}N$ enrichment is likely more related to the sources of N used for
332 growth (Atasoglu et al., 2001; Brito et al., 2006) and possibly the amount of time the bacteria
333 had to take up the label. The approach used in this experiment followed the concept of a plateau
334 in enrichment and cows were infused for 72 h before any measurements were made (Marini and
335 Van Amburgh, 2003; Recktenwald et al. 2014), thus different enrichment levels were likely not
336 due to non-plateau of $^{15}N$. Further, Recktenwald (2010) demonstrated that the rumen $NH_3$-N
337 pool reaches equilibrium rapidly after bolus dosing with doubly-labeled urea. In this study, it is
338 unlikely that relatively small changes in the ammonia pool, as reported in the companion paper,
339 would lead to bias in enrichment measurements due to $^{15}N$ dilution.

340 Total AA content in bacteria and protozoa was increased in cows fed EXP vs. CON. Amino
341 acid content as a percent of N was not different between diets, and averaged 50.3 and 54.0% for
342 omasal bacteria and protozoa, respectively. These values are lower than reported previously in
343 the literature (Storm and Ørskov, 1983). Hvelplund (1986) reported a mean AA N as a percent of
344 N of 67.4% in mixed rumen bacteria, and demonstrated a curvilinear relationship of diet starch
345 and sugar content vs. AA N as a percent of N, with AA N decreasing rapidly as starch+sugar
346 content exceeded 30% of diet DM. Reporting of AA N in this experiment did not include DAPA,
347 which can represent greater that 10% of the amino acid content in rumen micro-organisms.
348 Another explanation for the lower AA N content of the microbes could be related to the
349 procedure used in this experiment. Formalin has been previously shown to affect the AA
350 composition of isolated cells (Stern et al., 1983), likely through cross-linkage of protein chains.
351 Volden and Harstad (1998) reported an 11% decrease in total AA N as a percent of N when
352 formalin treatment was used in the isolation procedure, with some individual AA such as Lys,
353 Tyr, decreasing more than 30%. Additionally, the formalin treatment can create products

resistant even to acid hydrolysis, rendering incomplete extraction of AA from the sample matrix (Barry, 1976; Fessenden et al., 2017). Therefore, bacterial and protozoal AA profiles and contributions to AA flow might be underestimated in this study; however total flow of AA in OTD is unaffected, as no formalin was used in the separation of LQ, SP, or LP fractions. Overall, this data on AA N composition of microbes suggests the use of formalin in the isolation procedure should be eliminated if AA analysis and composition is an objective of the experiment.

Omasal bacteria and protozoa AA composition was unaffected by diet (Table 3). Profiles of bacterial amino acids generally agree well with literature reports (Volden et al., 1999) with the exception of Lys and Tyr, which were decreased in the current study and formalin is known to specifically affect these AA (Volden and Harstad, 1998). The protozoal AA profile was similar when compared with bacterial AA profile, with the exception of Met and Lys showing numerically increased levels in protozoa. Volden et al. (1999) also reported increased Lys concentration in protozoa vs. bacteria; however Met was very similar between isolates in that study. Cockburn and Williams (1984) reported mean Met concentrations of 2.4 g / 100 g AA which is very consistent with the average value of the two treatments in this study, 2.45 g / 100 g AA. Inconsistent use and poor reporting of pre-oxidation procedures used among studies makes many comparisons of sulfur AA difficult, as recoveries from pre-oxidation are rarely reported (Spindler et al., 1984). It is possible that formalin treatment increased the relative proportion of Met in our microbial isolates; however this effect should also be distributed across other AA not affected by formalin treatment.

***Omasal Flows of AA in OTD, Microbial, and Non-microbial Fractions***

Microbial nutrient flow of DM, OM and NAN flow was not different between diets (Table 4). Effects of rumen degradable protein source on omasal nutrient flows have been previously described in Fessenden et al (20XXa). Cows fed CON vs. EXP tended to have increased bacterial DM and OM flow (4808 vs. 4056 g DM/d and 4023 vs. 3433 g OM/d for CON vs. EXP, respectively). Since bacterial OM and DM flow are calculated using microbial N as a marker, it is likely that the observed difference in microbial N composition contributed to lower calculated DM and OM flows. Protozoa nutrient flows followed similar numeric trends, but flows did not differ significantly between diets. Protozoa accounted for 15.8 and 17.9% of the total microbial NAN flow in cows fed CON vs. EXP, respectively. This estimate is slightly higher than has previously been reported in the literature with animals at similar levels of intake. Sylvester et al. (2005) reported protozoa N accounting for 5.9 to 11.9% of the microbial N flow using 18S rDNA techniques, while Ahvenjärvi et al. (2002) reported 7% of microbial N flow as protozoa N using a very similar technique as our study; albeit with much different diets. Very few studies attempt to directly measure protozoa flows due to the difficulty of the approach. As such, some researchers have taken alternative approaches to estimate protozoal contribution to the microbial N pool. Using a linear programming approach, Shabi et al. (2000) estimated protozoal N to account for 7 to 19% of microbial N flow. This was a result similar to that estimated by Steinhour et al. (1982) using a differential $^{15}N$ enrichment approach, although many assumptions were made pertaining to pool size and turnover in that study. Alternatively, computer simulations by Dijkstra et al. (1998) indicated that protozoa N could account for 10.7 to 26.1% of microbial N in cattle at 17.1 kg of DMI. Simulations using CNCPS v. 7 indicated that overall microbial flow in cows fed CON was well predicted; however the model was insensitive to the numerical difference in microbial flow in CON vs. EXP fed cows (Table 4).

Protozoa flow (g/d) was underpredicted by 43%. It is possible that the coefficients used to calculate protozoa growth are relatively low as they are often based on in vitro studies of the more easily cultured protozoa species (Coleman and Hall, 1984; Coleman, 1992). The rates of substrate uptake and growth reported in vitro experiments might be considerably lower than those achieved rumen. It is also possible that the predictions of protozoa passage from the rumen are not correct in CNCPS v7 because there are few data on which to build robust equations and the model structure uses particle passage as a basis whereas, protozoa might be passing in the liquid phase, which would lead to underestimations in the current predictions. Future studies measuring microbial N flows should report protozoal contribution to the microbial flow, as data in this area are lacking.

The flow of AA in OTD is presented in Table 5. Most AA demonstrated increased flow in cows fed EXP vs. CON. Total AA flow was increased by 211 g/d in cows fed EXP compared to CON (2456 v. 2245 g/d for CON vs. EXP, respectively; $P < 0.01$). Omasal flow of Lys, Met, and Phe, were similar between diets, while all other EAA were increased in cows fed EXP compared with CON. Total non-essential AA flow was increased by 116 g/d in cows fed EXP, while Cys flow was the only individual NEAA that was similar between diets. Reynal and Broderick (2005) reported similar results in omasal AA flows when diets with varying RDP from soybean meal vs. treated soybean meal were fed to lactating dairy cows. When soybean meal, cottonseed meal, and canola meal were compared with a urea control, flows of all AA increased greatly in a study by Brito et al. (2007). The increase in AA flows in the current study was directly related to the lower dietary CP degradation in cows fed EXP, as reported by Fessenden et al. (20XXa). It is notable that while total NAN flow was not different between diets, total AA flow was increased in cows fed fermentation byproduct. This would suggest that the NAN flow in cows fed the CON

diets might have a higher content of non-AA nitrogenous compounds. The observed results might also occur if AA with higher N content are preferentially degraded in the rumen of cows fed fermentation byproduct, thus reducing the measured NAN flow. Overall, fermentation byproduct inclusion increased non-microbial AA flow relative to control. (Table 6). Inclusion of the fermentation byproduct had no effect on microbial AA flow, while non-microbial AA flow was increased for most individual AA. This further supports the protein sparing effect of the fermentation byproduct on RDP, as microbial AA flow was not significantly lower, while non-microbial AA flow increased by 316 g/d in cows fed EXP compared to CON ($P = 0.03$). Microbial protein synthesis was apparently not negatively affected by decreased CP degradation, indicating that sufficient AA and N compounds were present to support high rates of microbial growth. It should be noted that the non-microbial AA flows presented in Table 6 are calculated by subtraction of microbial AA flow from total AA flow. As such, experimental and measurement errors will be disproportionally represented in the reported non-microbial AA flows. This might also lead to values for individual AA that are outside biologically expected ranges. This occurred with Trp, where the flow (determined by difference of total AA flow vs. microbial AA flow) would result in a negative number.

***Omasal Flows of AA in Bacteria and Protozoa***

Flows of individual amino acids in bacterial and protozoa fractions were generally not affected by diet (Table 7). Bacterial Leu flow was increased in cows fed CON vs. EXP (63.4 vs. 45.1 g/d for CON vs. EXP, respectively; $P = 0.03$). Bacterial Ser and Tyr flows tended to be increased in cows fed CON vs. EXP, while all other bacterial AA flows were not different between diets. Protozoa AA flows were unaffected by treatment. The contribution of protozoa to total microbial AA flow is in Table 8. Inclusion of the fermentation byproduct in the EXP diets

increased protozoa contribution to Leu and Lys flow. Protozoa flow of Lys accounted for 21.5 vs. 29.5% of the total microbial flow of Lys in cows fed EXP vs. CON, respectively, ($P < 0.01$). Contribution of protozoa to total EAA flow tended to be greater in cows fed EXP vs. CON (19.0 vs. 22.8% of microbial flow, respectively; $P = 0.07$). These results demonstrate the importance of protozoa to post ruminal AA flows. Protozoa NAN contribution to microbial NAN flows averaged 16.9% of microbial N, while contribution of protozoa AA to microbial AA flows ranged from 8 to 46% for individual AA. Models that do not take into account the difference in AA profile, composition, and contribution of protozoa to microbial AA flow might have poor predictions of post-ruminal AA flow. For models seeking to describe the diet adequacy to support milk protein production, accurate predictions of post ruminal supply are needed if the models are to be applied in practical feeding situations (Pacheco et al., 2012). Higgs (2014) evaluated CNCPS v. 7 against a large literature dataset, and found the model adequately predicts post ruminal total NAN and microbial NAN supply; however individual AA were generally over-predicted. The same tendency to over-predict AA flow was observed in the current evaluation might be indicative of mean bias of the model, or could be related to a methodological bias associated with the use of the triple-marker system, as all studies in the dataset utilized this approach.

***Rumen Pool Size and Kinetics of OM, CHO, Bacteria and Protozoa***

Rumen pool sizes of digestible OM, CHO, NAN, and microbial NAN were not affected by diet (Table 9). Generally, microbial N pool size of 3.5 g of microbial N/L was similar to that observed by Sylvester et al. (2005), who reported a microbial pool of approximately 3.0 g of microbial N/L, although the authors point out that the estimation of bacterial N contains the error of both the 18S RNA procedure and the purine determination. Purines have been shown to be

468 inconsistent microbial markers relative to $^{15}N$ (Klopfenstein et al., 2001; Firkins and Reynolds,
469 2005; Ipharraguerre et al., 2007). Cows fed CON had greater microbial DM contribution to the
470 total DM pool than cows fed EXP (27.7 vs. 23.6 % for CON vs. EXP, respectively; $P = 0.05$).
471 The values in this experiment were within the range of 17 to 27 % of the rumen DM pool as
472 microbial DM reported by Craig et al. (1987).

473 Selective retention of protozoa in the rumen is not well understood, and estimates range from
474 < 5 % (Sylvester et al., 2005) to over 70% (Punia et al., 1992). Since rumen protozoa mass was
475 not quantified directly in this study, the effect of 4 different levels of retention is described in
476 Table 9. Further, since total rumen microbial pool size was estimated from $^{15}N$ enrichment of the
477 rumen NAN pool, it is possible to evaluate the effect of selective retention on pool sizes,
478 assuming total microbial $^{15}N$ pool size remains constant. Therefore, at 0% selective retention, we
479 expect the protozoa to account for the same proportion of total rumen microbial N as measured
480 in OTD flow, while at greater levels of retention, protozoa account for larger portions of the
481 microbial pool. Bacteria pool size was decreased in cows fed EXP vs. CON, and bacteria vs.
482 protozoa pool sizes diverged as estimated selective retention increased. At the highest estimation
483 of selective retention, protozoa were calculated to represent 55 to 58% of the total microbial pool
484 (CON vs. EXP, respectively, $P = 0.40$).

485 To assess which level of selective retention of protozoa is likely most correct, it was possible
486 to use pool size and flow to estimate fractional rates of turnover (Table 10). In this case, since
487 actual flows and microbial pool size were measured, the rate of turnover of the pool can be used
488 as an index of microbial growth rate (Wells and Russell, 1996). Recognizing that the main
489 energy substrate for rumen bacteria is CHO (Russell et al., 1992), and assuming the maximum
490 yield of cell DM per g of CHO degraded is 0.5 (Isaacson et al., 1975), one can quickly determine

491 which retention values allows for realistic growth rates. In this instance, selective retention at
492 50% indicate that bacteria would have to grow at a fractional rate of 0.07 $h^{-1}$, corresponding to a
493 CHO degradation rate of 0.14 $h^{-1}$ (0.07 / 0.5). Given the estimated pool size (g) and digestion
494 (g/h), the fractional rate of CHO availability in this study averaged 0.138 $h^{-1}$ of the available
495 pool; therefore theoretical maximal fractional growth rate was estimated at 0.138 x 0.5, or ~
496 0.069 $h^{-1}$. Using the measured total microbial pool at 25% selective retention, it was calculated
497 that the fractional growth rate of all microbes in the rumen was 0.061 $h^{-1}$. This corresponds to an
498 estimated $Y_g$ of 0.44 g/g of CHO degraded. This value is close to the theoretical maximums for
499 individual species reported in pure cultures (Russell and Baldwin, 1979; Theodorou and France,
500 2005). In vitro measurements of mixed rumen microbes often give yields on the high range of
501 those observed in pure culture (Russell and Wallace, 1997). The range of yields (29 to 100 g /
502 mol of hexose) reported by Russell and Wallace (1997), correspond to $Y_g$ of 0.16 to 0.55 g / g of
503 glucose degraded. Stouthamer (1973) estimated a maximal $Y_g$ of approximately 0.8 g / g of
504 glucose using biochemical pathways, indicating the possibility for much higher yields in some
505 bacterial species; however values this high are rarely reported in vitro with mixed rumen
506 microbial fermentations. It is possible that higher growth rates could be achieved in vivo, as it
507 can be very difficult to maintain ideal conditions for microbial growth outside the rumen.

508 The CNCPS relates cell growth directly to CHO availability in the manner described above,
509 so accurate estimates of CHO degradation are key to accurately predicting microbial yield.
510 Simulations indicated that the model characterized CHO digestion reasonably well (Table 10)
511 with predicted absolute rates of degradation approximately 37 grams lower than observed. This
512 corresponds to a fractional rate of degradation of CHO at 0.124 $h^{-1}$. Given the predicted
513 microbial yield (Table 4), the apparent $Y_g$ used by the model was 0.45 g / g of CHO degraded in

514 the rumen; very similar to the value observed in vivo. Unfortunately, many other in vivo studies
515 investigating rumen outflows do not report rumen pool sizes or individual microbial populations,
516 thereby limiting the utility of the data for model evaluation. Even so, the limited data presented
517 here shows good agreement of predicted vs. independent measured values, indicating that the
518 structure of the model is likely adequate to provide accurate estimates of microbial yield from
519 substrate degradation. This provides a strong basis from which to improve AA supply
520 predictions, as microbial AA represents a large portion of metabolizable AA flowing from the
521 rumen.

522 **CONCLUSIONS**

523 The inclusion of the fermentation byproduct in EXP diets increased microbial N and AA N
524 flows compared with CON cows fed a urea control. Microbial AA composition did not differ
525 between diets; however estimates of total AA N and specific AA were likely lower than
526 literature values due to formalin treatment. Daily flows of AA were increased in OTD as a result
527 of decreased degradation of feed N as reported by Fessenden et al. (20XXa). This was reflected
528 in the current study with increased non-microbial AA flow in cows fed EXP vs. CON. Average
529 protozoa contribution to microbial NAN flow was 16.8%, yet protozoa AA accounted for 21% of
530 the microbial EAA flow, with a range of 8 to 46% for individual AA. Cows in this study
531 maintained an average pool size of 320 g of microbial N in the rumen, while bacterial and
532 protozoal pools were estimated at 4 different theoretical levels of retention. Fractional growth
533 rate of all microbes in the rumen was measured at 0.069 $h^{-1}$, with a Yg of 0.44 g / g of CHO
534 degraded. A dynamic versions of the CNCPS v. 7 was able to accurately predict CHO
535 degradation and total microbial yield, however improvements are needed for bacteria vs.
536 protozoa partitioning and individual AA flow predictions. Overall, the current structure of the

CNCPS v. 7 provides a strong base for predicting supply of microbial NAN. Future model improvements in microbial AA profiles, intestinal digestibility, protozoa partitioning, and dietary protein degradation might be needed to improve estimates of individual AA flow.

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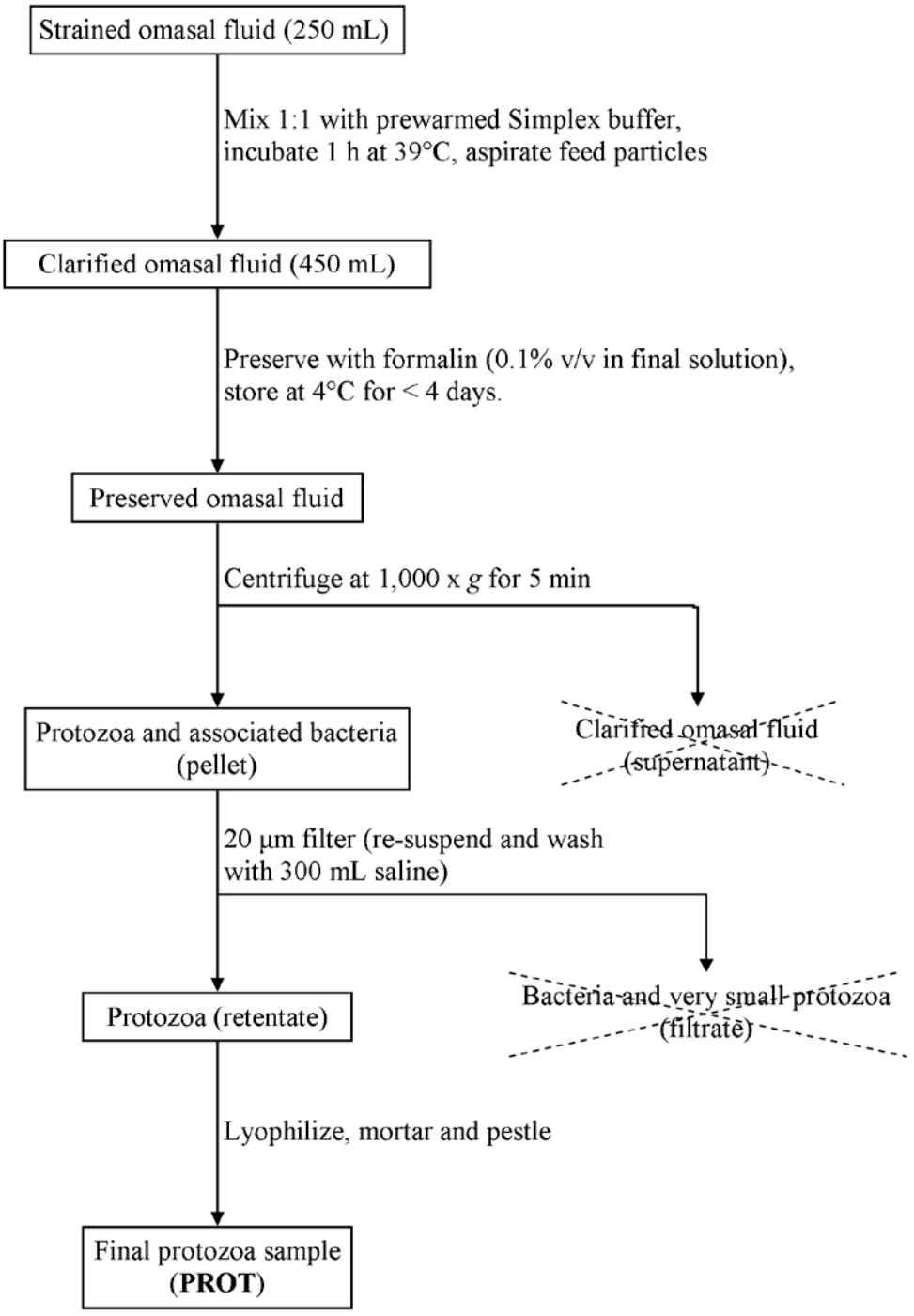
**Table 1.** Ingredient and nutrient composition (mean ± SD)[1] of experimental diets

| | Diet | |
|---|---|---|
| Item | CON | EXP |
| Ingredient, % DM | | |
| Corn silage | 44.6 | 44.6 |
| Alfalfa silage | 12.0 | 12.0 |
| Corn meal | 12.0 | 12.0 |
| Expeller soybean meal[2] | 8.0 | 8.0 |
| Soybean hulls | 5.8 | 5.8 |
| Citrus pulp, dry | 3.3 | 3.3 |
| Chocolate meal | 2.4 | 2.4 |
| Saturated fatty acid[3] | 1.2 | 1.2 |
| Molasses | 0.9 | 0.9 |
| Blood meal | 1.7 | 1.7 |
| Wheat middlings | 4.8 | 3.2 |
| Fermentation byproduct[4] | – | 3.0 |
| Calcium carbonate | – | 0.7 |
| Urea | 0.4 | – |
| Calcium sulfate, dihydrate | 1.7 | – |
| Sodium bicarbonate | 0.33 | 0.40 |
| Salt white | 0.30 | 0.32 |
| Magnesium oxide | 0.17 | 0.17 |
| Dicalcium phosphate | 0.16 | 0.16 |
| Supplemental methionine[5] | 0.06 | 0.06 |
| Vitamin and mineral mix[6] | 0.18 | 0.18 |
| Nutrient composition | | |
| DM, % | 44.5 ± 0.7 | 44.2 ± 0.8 |
| OM, % of DM | 93.9 ± 0.3 | 93.8 ± 0.6 |
| CP, % of DM | 15.9 ± 0.6 | 16.1 ± 0.5 |
| RDP, % of DM[7] | 8.4 ± 0.1 | 8.0 ± 0.1 |
| Starch, % of DM | 27.5 ± 1.1 | 27.8 ± 0.5 |
| Sugars, % of DM | 5.4 ± 0.4 | 5.3 ± 0.3 |
| NFC, % of DM[7] | 41.7 ± 0.2 | 41.8 ± 1.3 |
| aNDFom, % of DM | 30.9 ± 0.2 | 31.2 ± 0.2 |
| ADF, % of DM | 19.9 ± 1.5 | 19.7 ± 0.6 |
| ADL, % of NDF | 10.0 ± 0.9 | 10.0 ± 1.4 |
| Ether extract, % of DM | 5.0 ± 0.2 | 4.9 ± 0.2 |
| ME, Mcal/kg[7] | 2.5 ± 0.1 | 2.5 ± 0.1 |

[1]Analyzed values from 3 period composite samples. Table is from Fessenden et al. (20XXa)
[2]SOYPLUS (West Central Cooperative, Ralston, IA).
[3]ENERGY BOOSTER 100 (MSC Company, Dundee, IL).
[4]FERMENTEN (Church & Dwight, Inc., Princeton, NJ).
[5]SMARTAMINE M (Bluestar Adisseo Nutrition Group, Alpharetta, GA).
[6]Provided (per kg of diet DM): 44 mg of Zn, 32 mg of Mn, 10 mg of Cu, 1 mg of Co, 1 mg of I, 0.3 mg of Se, 5000 IU of vitamin A, 980 IU of vitamin B, and 25 IU of vitamin E.
[7]Calculated by the Cornell Net Carbohydrate and Protein System v. 6.5.

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765 **Figure 3.1. Flowchart for preparation of protozoa isolates**. Fractions discarded are crossed

766 out.

**Table 2.** Chemical composition and isotopic enrichment of omasal bacteria and protozoa in lactating dairy cattle fed two different sources of rumen available nitrogen

| | Diet[1] | | | |
|---|---|---|---|---|
| Item | CON | EXP | SEM | *P* |
| Bacteria | | | | |
| OM, % of DM | 83.6 | 84.7 | 0.5 | 0.18 |
| N, % of DM | 7.90 | 8.29 | 0.13 | 0.02 |
| N, % of OM | 9.45 | 9.79 | 0.14 | 0.04 |
| $^{15}$N atom% excess | 0.035 | 0.034 | 0.002 | 0.74 |
| Total AA, % of DM | 28.8 | 30.8 | 0.4 | 0.01 |
| Total AA, % of OM | 34.3 | 36.3 | 0.4 | 0.02 |
| AA, % of N | 50.1 | 50.6 | 0.8 | 0.67 |
| Protozoa | | | | |
| OM, % of DM | 86.1 | 89.1 | 1.5 | 0.17 |
| N, % of DM | 7.93 | 8.60 | 0.24 | 0.05 |
| N, % of OM | 9.21 | 9.65 | 0.24 | 0.24 |
| $^{15}$N atom% excess | 0.023 | 0.020 | 0.002 | 0.11 |
| Total AA, % of DM | 31.4 | 34.4 | 0.9 | 0.02 |
| Total AA, % of OM | 36.4 | 38.6 | 0.9 | 0.12 |
| AA, % of N | 53.4 | 54.6 | 0.9 | 0.32 |

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

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**Table 3.** Omasal bacteria and protozoa amino acid composition in lactating dairy cattle fed two different sources of rumen available nitrogen

| | Bacteria AA, g/100 g AA | | | | Protozoa AA, g/100 g AA | | | |
|---|---|---|---|---|---|---|---|---|
| | Diet[1] | | | | Diet[1] | | | |
| Item | CON | EXP | SEM | *P* | CON | EXP | SEM | *P* |
| Essential AA | | | | | | | | |
| ARG | 5.60 | 5.38 | 0.23 | 0.49 | 5.16 | 5.84 | 0.36 | 0.20 |
| HIS | 1.96 | 2.02 | 0.04 | 0.30 | 2.67 | 2.67 | 0.12 | 0.96 |
| ILE | 4.70 | 4.66 | 0.06 | 0.60 | 5.15 | 4.91 | 0.25 | 0.51 |
| LEU | 4.42 | 3.60 | 0.30 | 0.07 | 5.59 | 5.61 | 0.40 | 0.96 |
| LYS | 4.13 | 4.04 | 0.05 | 0.24 | 6.15 | 6.16 | 0.26 | 0.98 |
| MET | 2.34 | 2.56 | 0.16 | 0.34 | 3.99 | 4.00 | 0.10 | 0.93 |
| PHE | 6.50 | 6.44 | 0.15 | 0.79 | 7.08 | 6.85 | 0.14 | 0.29 |
| TRP | 4.94 | 4.98 | 0.13 | 0.85 | 3.74 | 3.63 | 0.09 | 0.26 |
| THR | 6.08 | 5.93 | 0.21 | 0.62 | 4.79 | 4.90 | 0.25 | 0.75 |
| VAL | 6.55 | 6.71 | 0.09 | 0.16 | 5.83 | 6.05 | 0.22 | 0.49 |
| Total EAA | 47.2 | 46.3 | 0.6 | 0.26 | 50.1 | 50.8 | 0.4 | 0.23 |
| | | | | | | | | |
| Nonessential AA | | | | | | | | |
| ALA | 7.07 | 7.18 | 0.08 | 0.36 | 5.66 | 5.62 | 0.17 | 0.86 |
| ASP | 10.65 | 10.67 | 0.32 | 0.95 | 11.45 | 11.87 | 0.67 | 0.62 |
| CYS | 1.20 | 1.14 | 0.06 | 0.49 | 2.84 | 2.80 | 0.05 | 0.62 |
| GLU | 14.36 | 14.59 | 0.15 | 0.30 | 13.87 | 14.11 | 0.28 | 0.49 |
| GLY | 5.77 | 5.89 | 0.05 | 0.10 | 4.84 | 4.84 | 0.13 | 0.99 |
| PRO | 7.22 | 8.57 | 0.46 | 0.04 | 3.96 | 2.73 | 0.95 | 0.37 |
| SER | 5.31 | 4.81 | 0.20 | 0.10 | 4.49 | 4.74 | 0.20 | 0.40 |
| TYR | 1.15 | 0.82 | 0.16 | 0.02 | 2.72 | 2.60 | 0.20 | 0.67 |
| Total NEAA | 52.8 | 53.7 | 0.60 | 0.26 | 49.9 | 49.2 | 0.40 | 0.23 |

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

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**Table 4.** Omasal microbial nutrient flows and protozoa predation in lactating dairy cattle fed two different sources of rumen available nitrogen

| | Diet[1] | | | |
|---|---|---|---|---|
| Item | CON | EXP | SEM | *P* |
| Dry matter intake, kg/d[2] | 23.8 | 23.9 | 0.7 | 0.91 |
| | | | | |
| Total microbial nutrient flow, g/d | | | | |
| DM flow | 5,718 | 4,930 | 358 | 0.14 |
| OM flow | 4,815 | 4,210 | 310 | 0.19 |
| NAN flow[2] | 450 | 409 | 28 | 0.31 |
| | | | | |
| Bacterial nutrient flow, g/d | | | | |
| DM flow | 4,808 | 4,056 | 286 | 0.08 |
| OM flow | 4,023 | 3,433 | 240 | 0.10 |
| NAN flow | 378 | 337 | 23.0 | 0.22 |
| % of microbial N flow | 84.2 | 82.1 | 1.0 | 0.12 |
| | | | | |
| Protozoa nutrient flow, g/d | | | | |
| DM flow | 909 | 850 | 82 | 0.61 |
| OM flow | 790 | 764 | 81 | 0.82 |
| NAN flow | 72.1 | 73.9 | 7.3 | 0.84 |
| % of microbial N flow | 15.8 | 17.9 | 1.0 | 0.12 |
| | | | | |
| Protozoa predation of bacteria, g/d | | | | |
| DM consumed | 1,159 | 929 | 166 | 0.33 |
| OM consumed | 967 | 783 | 138 | 0.35 |
| N consumed | 90.6 | 76.3 | 12.9 | 0.45 |
| % of bacterial N flow | 23.4 | 22.2 | 2.4 | 0.70 |
| | | | | |
| CNCPS v. 7 output | | | | |
| Predicted microbial N flow, g/d | 412 | 417 | - | - |
| Bacteria N flow | 371 | 375 | - | - |
| Protozoa N flow | 41 | 42 | - | - |
| % of microbial N flow | 9.9 | 10.1 | - | - |
| Predation estimate, bacterial N consumed, g/d | 75 | 76 | - | - |

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.
[2]Previously reported in Fessenden et al. (20XXa)

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**Table 5.** Effect of rumen available nitrogen source on omasal true digesta flow of AA in lactating dairy cattle

| | Diet[1] | | | |
|---|---|---|---|---|
| AA flow, g/d | CON | EXP | SEM | *P* |
| Essential AA | | | | |
| ARG | 108.2 | 120.1 | 3.4 | 0.01 |
| HIS | 62.2 | 68.0 | 1.5 | < 0.01 |
| ILE | 87.3 | 100.2 | 2.7 | < 0.01 |
| LEU | 131.0 | 149.2 | 7.0 | 0.03 |
| LYS | 139.0 | 127.4 | 14.0 | 0.56 |
| MET | 57.3 | 57.3 | 5.5 | 1.00 |
| PHE | 147.7 | 165.8 | 8.3 | 0.11 |
| TRP | 78.9 | 86.3 | 2.4 | < 0.01 |
| THR | 119.9 | 131.6 | 3.3 | < 0.01 |
| VAL | 116.8 | 131.1 | 3.6 | < 0.01 |
| Total EAA | 1,045.8 | 1,141.8 | 28.4 | 0.03 |
| | | | | |
| Nonessential AA | | | | |
| ALA | 140.9 | 153.0 | 4.0 | < 0.01 |
| ASP | 212.3 | 236.2 | 5.7 | < 0.01 |
| CYS | 39.3 | 38.0 | 3.4 | 0.78 |
| GLU | 280.1 | 314.0 | 6.3 | < 0.01 |
| GLY | 105.9 | 116.5 | 2.7 | < 0.01 |
| PRO | 172.1 | 189.4 | 6.2 | 0.03 |
| SER | 120.0 | 131.4 | 3.3 | < 0.01 |
| TYR | 130.4 | 138.7 | 4.4 | 0.05 |
| Total NEAA | 1,200.9 | 1,316.8 | 31.4 | < 0.01 |
| | | | | |
| Total AA | 2,245.2 | 2,456.5 | 57.1 | < 0.01 |

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

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**Table 6.** Effect of rumen available nitrogen source on omasal flow of microbial and non-microbial AA in lactating dairy cattle

| | Microbial AA flow, g/d | | | | Non-microbial AA flow, g/d | | | |
|---|---|---|---|---|---|---|---|---|
| | Diet[1] | | | | Diet[1] | | | |
| Item | CON | EXP | SEM | *P* | CON | EXP | SEM | *P* |
| Essential AA | | | | | | | | |
| ARG | 96.9 | 88.4 | 6.7 | 0.38 | 10.7 | 31.8 | 7.9 | 0.08 |
| HIS | 36.4 | 34.7 | 2.3 | 0.57 | 25.8 | 33.2 | 2.7 | 0.04 |
| ILE | 83.1 | 75.4 | 5.7 | 0.34 | 4.2 | 23.4 | 6.4 | 0.01 |
| LEU | 79.5 | 61.7 | 6.3 | 0.06 | 52.9 | 87.9 | 9.7 | 0.02 |
| LYS | 78.4 | 71.9 | 5.8 | 0.42 | 60.5 | 56.3 | 14.7 | 0.84 |
| MET | 43.1 | 44.8 | 3.4 | 0.67 | 14.0 | 12.8 | 6.1 | 0.88 |
| PHE | 114.0 | 103.0 | 7.8 | 0.35 | 33.4 | 61.9 | 10.4 | 0.06 |
| TRP | 82.7 | 74.5 | 5.2 | 0.28 | − | 11.4 | 6.7 | − |
| THR | 103.3 | 92.9 | 7.8 | 0.35 | 16.4 | 37.0 | 8.9 | 0.05 |
| VAL | 112.4 | 106.3 | 8.0 | 0.58 | 4.3 | 23.1 | 10.0 | 0.09 |
| Total EAA | 830.2 | 749.0 | 51.4 | 0.28 | 213.6 | 371.7 | 64.6 | 0.04 |
| Nonessential AA | | | | | | | | |
| ALA | 119.8 | 111.2 | 8.0 | 0.43 | 20.5 | 40.5 | 9.7 | 0.07 |
| ASP | 188.7 | 177.8 | 13.8 | 0.51 | 23.1 | 56.7 | 15.5 | 0.04 |
| CYS | 24.8 | 23.0 | 1.5 | 0.34 | 14.4 | 15.3 | 3.7 | 0.86 |
| GLU | 249.8 | 234.9 | 17.7 | 0.51 | 30.0 | 76.7 | 19.9 | 0.04 |
| GLY | 98.1 | 91.7 | 6.8 | 0.48 | 7.6 | 23.7 | 7.8 | 0.08 |
| PRO | 117.0 | 122.1 | 9.6 | 0.66 | 55.7 | 65.8 | 12.7 | 0.42 |
| SER | 90.5 | 79.5 | 6.7 | 0.17 | 29.5 | 50.7 | 8.3 | < 0.01 |
| TYR | 24.2 | 19.4 | 2.5 | 0.09 | 106.2 | 119.1 | 4.4 | < 0.01 |
| Total NEAA | 913.1 | 861.0 | 61.7 | 0.49 | 287.1 | 445.6 | 73.7 | 0.03 |
| Total AA | 1,744.2 | 1,611.4 | 113.9 | 0.39 | 500.0 | 815.9 | 135.4 | 0.03 |

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

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**Table 7.** Effect of rumen available nitrogen source on omasal flow of bacteria and protozoa AA flow in lactating dairy cattle

| | Bacteria AA flow, g/d | | | | Protozoa AA flow, g/d | | | |
|---|---|---|---|---|---|---|---|---|
| | Diet[1] | | | | Diet[1] | | | |
| Item | CON | EXP | SEM | *P* | CON | EXP | SEM | *P* |
| Essential AA | | | | | | | | |
| ARG | 81.8 | 70.5 | 5.9 | 0.20 | 15.3 | 18.4 | 2.5 | 0.35 |
| HIS | 28.6 | 26.5 | 2.0 | 0.45 | 7.8 | 8.0 | 0.6 | 0.77 |
| ILE | 68.0 | 60.2 | 4.7 | 0.27 | 15.0 | 15.1 | 1.6 | 0.95 |
| LEU | 63.4 | 45.1 | 5.4 | 0.03 | 16.1 | 16.6 | 1.4 | 0.79 |
| LYS | 60.2 | 53.4 | 4.3 | 0.26 | 18.1 | 18.6 | 1.9 | 0.85 |
| MET | 31.5 | 32.5 | 2.8 | 0.77 | 11.7 | 12.1 | 1.2 | 0.84 |
| PHE | 93.3 | 82.5 | 6.4 | 0.25 | 20.6 | 20.7 | 1.9 | 0.96 |
| TRP | 71.8 | 63.5 | 4.7 | 0.23 | 10.9 | 11.0 | 1.0 | 0.91 |
| THR | 89.0 | 77.4 | 6.4 | 0.21 | 14.2 | 15.1 | 1.9 | 0.71 |
| VAL | 95.1 | 87.2 | 6.5 | 0.41 | 17.1 | 18.6 | 2.2 | 0.60 |
| Total EAA | 682.9 | 597.1 | 42.2 | 0.17 | 146.9 | 154.1 | 14.8 | 0.71 |
| Nonessential AA | | | | | | | | |
| ALA | 103.1 | 93.7 | 6.9 | 0.34 | 16.6 | 17.1 | 1.9 | 0.86 |
| ASP | 154.8 | 141.7 | 10.6 | 0.31 | 34.0 | 36.2 | 4.6 | 0.69 |
| CYS | 16.6 | 14.4 | 1.1 | 0.17 | 8.3 | 8.4 | 0.7 | 0.93 |
| GLU | 208.8 | 191.5 | 14.9 | 0.38 | 40.9 | 42.9 | 4.3 | 0.70 |
| GLY | 83.8 | 76.4 | 5.7 | 0.38 | 14.3 | 14.9 | 1.7 | 0.77 |
| PRO | 105.5 | 114.0 | 10.3 | 0.50 | 11.4 | 8.2 | 2.4 | 0.37 |
| SER | 77.2 | 64.5 | 5.7 | 0.10 | 13.3 | 14.3 | 1.7 | 0.66 |
| TYR | 16.1 | 11.2 | 2.5 | 0.06 | 8.1 | 8.0 | 1.0 | 0.94 |
| Total NEAA | 766.1 | 708.6 | 51.5 | 0.39 | 147.1 | 149.5 | 15.0 | 0.90 |
| Total AA | 1,449.7 | 1,304.3 | 93.6 | 0.27 | 293.9 | 303.7 | 29.8 | 0.80 |

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

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**Table 8.** Effect of rumen available nitrogen source on protozoa proportion of omasal microbial AA flow in lactating dairy cattle

| | Diet[1] | | | |
|---|---|---|---|---|
| Protozoa AA contribution to microbial AA flow, % | CON | EXP | SEM | *P* |
| Essential AA | | | | |
| ARG | 17.0 | 22.0 | 2.0 | 0.07 |
| HIS | 22.5 | 25.9 | 1.7 | 0.18 |
| ILE | 18.8 | 21.5 | 1.6 | 0.15 |
| LEU | 21.5 | 29.5 | 1.6 | < 0.01 |
| LYS | 23.9 | 28.2 | 1.6 | 0.05 |
| MET | 28.6 | 31.3 | 2.5 | 0.46 |
| PHE | 19.0 | 22.0 | 1.7 | 0.22 |
| TRP | 17.8 | 24.4 | 4.1 | 0.27 |
| THR | 14.7 | 17.8 | 1.5 | 0.15 |
| VAL | 16.0 | 19.0 | 1.4 | 0.17 |
| Total EAA | 19.0 | 22.8 | 1.5 | 0.07 |
| | | | | |
| Nonessential AA | | | | |
| ALA | 14.7 | 17.1 | 1.4 | 0.25 |
| ASP | 18.9 | 22.6 | 1.8 | 0.09 |
| CYS | 34.4 | 40.0 | 2.4 | 0.11 |
| GLU | 17.2 | 20.1 | 1.5 | 0.15 |
| GLY | 15.2 | 17.8 | 1.5 | 0.23 |
| PRO | 10.6 | 7.7 | 2.7 | 0.46 |
| SER | 15.6 | 21.0 | 1.7 | 0.04 |
| TYR | 35.4 | 45.8 | 5.5 | 0.21 |
| Total NEAA | 16.8 | 19.4 | 1.3 | 0.20 |
| | | | | |
| Total AA | 17.8 | 20.9 | 1.4 | 0.13 |

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

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**Table 9.** Effect of rumen available nitrogen source on rumen pool sizes of organic matter, carbohydrate, and non-ammonia nitrogen in lactating dairy cattle

| | Diet[1] | | | |
|---|---|---|---|---|
| Item | CON | EXP | SEM | *P* |
| Rumen pool sizes | | | | |
| Digestible OM, kg[2] | 6.61 | 7.06 | 0.50 | 0.50 |
| Total fermentable CHO, kg[3] | 3.98 | 4.10 | 0.32 | 0.79 |
| Total NAN, g | 586 | 614 | 38 | 0.53 |
| Microbial NAN pool at 25% selective retention, g | 340 | 300 | 21 | 0.21 |
| Microbial DM proportion of rumen DM pool, % | 27.7 | 23.6 | 1.4 | 0.05 |
| Bacteria NAN rumen pool sizes, g[4] | | | | |
| 0% selective retention | 281 | 240 | 18 | 0.13 |
| 25% selective retention | 271 | 229 | 17 | 0.11 |
| 50% selective retention | 250 | 209 | 17 | 0.07 |
| 75% selective retention | 181 | 148 | 17 | 0.07 |
| Protozoa NAN rumen pool sizes, g[5] | | | | |
| 0% selective retention | 53 | 53 | 5 | 0.98 |
| 25% selective retention | 70 | 70 | 7 | 0.98 |
| 50% selective retention | 105 | 105 | 10 | 0.98 |
| 75% selective retention | 210 | 211 | 21 | 0.98 |
| Protozoa NAN pool, % of total microbial NAN pool | | | | |
| 0% selective retention | 15.8 | 17.9 | 1.0 | 0.12 |
| 25% selective retention | 20.7 | 23.2 | 1.3 | 0.13 |
| 50% selective retention | 30.1 | 33.1 | 1.8 | 0.17 |
| 75% selective retention | 55.1 | 58.0 | 2.9 | 0.40 |

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.
[2]Measured OM from rumen evacuation, corrected for microbial OM and uNDF240
[3]Rumen OM pool – (Rumen CP pool – Microbial CP pool) – (rumen DM pool * diet fat content)
[4]Microbial NAN pool – Protozoa NAN pool at 4 levels of selective retention of protozoa
[5]Microbial NAN x Protozoa % of omasal flow x level of selective retention

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**Table 10.** Fractional rates of microbial growth, nutrient digestion, and rumen fermentation parameters in lactating dairy cattle fed two different sources of rumen available nitrogen

| | Diet[1] | | | |
|---|---|---|---|---|
| Item | CON | EXP | SEM | *P* |
| Fractional growth rate of bacteria[2], $h^{-1}$ | | | | |
| 0% selective retention | 0.061 | 0.061 | 0.004 | 0.99 |
| 25% selective retention | 0.064 | 0.064 | 0.005 | 0.99 |
| 50% selective retention | 0.070 | 0.070 | 0.006 | 1.00 |
| 75% selective retention | 0.108 | 0.103 | 0.012 | 0.74 |
| Fractional growth rate of protozoa[2], $h^{-1}$ | | | | |
| 0% selective retention | 0.061 | 0.061 | 0.004 | 0.99 |
| 25% selective retention | 0.046 | 0.046 | 0.003 | 0.99 |
| 50% selective retention | 0.030 | 0.030 | 0.002 | 0.99 |
| 75% selective retention | 0.015 | 0.015 | 0.001 | 0.99 |
| Omasal flows and ruminal digestion parameters | | | | |
| True OM flow, kg/d[3] | 7.08 | 7.19 | 0.47 | 0.87 |
| Microbial NAN flow, g/d[3] | 450 | 409 | 28 | 0.31 |
| Ruminal true OM digestion rate, g/h | 626 | 619 | 17 | 0.77 |
| Ruminal true CHO digestion rate, g/h | 518 | 526 | 15 | 0.72 |
| Fractional rate of OM digestion[4], $h^{-1}$ | 0.101 | 0.094 | 0.008 | 0.54 |
| Fractional rate of CHO digestion[4], $h^{-1}$ | 0.139 | 0.138 | 0.011 | 0.91 |
| Microbial growth parameters | | | | |
| Fractional growth rate, $h^{-1}$ | 0.060 | 0.060 | 0.004 | 0.94 |
| Theoretical maximum CHO allowable growth[5], $h^{-1}$ | 0.070 | 0.069 | 0.005 | 0.91 |
| Observed Yg, g of cells / g of CHO degraded[6] | 0.44 | 0.44 | 0.03 | 0.99 |
| % of theoretical maximum Yg | 88.4 | 88.3 | 6.6 | 0.99 |
| CNCPS v. 7 output | | | | |
| Predicted CHO degradation, g/h | 484 | 487 | - | - |
| Predicted fractional rate of CHO digestion, $h^{-1}$ | 0.124 | 0.124 | - | - |
| Predicted Yg, g of cells / g of CHO degraded | 0.45 | 0.45 | - | - |

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.
[2]bacteria or protozoa daily flow (g/h) / bacteria or protozoa pool size (g) at 4 levels of protozoa selective retention
[3]Previously reported in Fessenden et al. (20XXa)
[4]Measured microbial NAN flow (g/h) / measured rumen microbial NAN pool (g)
[5]Fractional rate of CHO digestion x 0.5
[6]Fractional microbial growth rate / fractional rate of CHO digestion

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